to study cartilage matrix are currently (i) dGEMRIC (delayed gadolinium enhanced magnetic resonance imaging of cartilage), (ii) T2 relaxation time measurements and (iii) T1rho mapping techniques. These techniques have shown merit in determining the amount of macromolecules, such as glycosaminoglycans, within the cartilage and quantifying its water content.
In summary MRI's role in cartilage imaging is evolving with new diagnostic requirements; while assessment of cartilage morphology is still standard, innovative techniques to quantify matrix changes at early stages will be increasingly important in the future to tailor therapies and better understand disease progression on a molecular level.

17

NOVEL MEDIATORS OF CHONDROCYTE GENE EXPRESSION IN DEVELOPMENT AND OSTEOARTHRITIS

Mary B. Goldring

The human adult articular chondrocyte is a unique cell type that has reached a terminally differentiated state within the cartilage matrix as an end-point of development. In cartilage of patients with osteoarthritis (OA), the chondrocytes may undergo phenotypic modulation in response to alterations in the environment due to the mechanical loading and inflammation. Isolated chondrocytes in primary culture have provided useful models to study cellular responses to alterations in the environment such as those occurring in different forms of arthritis [1]. Immortalized chondrocytes of human origin have been developed to serve as reproducible models for studying transcriptional regulation of chondrocyte function by inflammatory mediators such as interleukin-1β (IL-1β) [2]. We reported previously that activation of EGR-1 by IL-1β results in early, transient suppression of proximal COL2A1 promoter activity by displacement of Sp1 from GC boxes [3]. The epithelium-specific ETS (ESE)-1 is expressed predominantly in epithelial cells but can also be induced in other cell types including chondrocytes in response to inflammatory mediators such as IL-1β and upregulates IL-1-induced genes, including COX-2 [4,5]. ESE-1 is a potent transcriptional suppressor of *COL2A1* promoter activity in chondrocytes, accounting for the sustained, NF-κB-dependent inhibition by IL-1β, and acts by interfering with the activator functions of CBP/300 and Sox9 [6]. Our finding that ESE-1 protein is localized intracellularly in chondrocytes in cartilage from patients with osteoarthritis but not from individuals with normal joints and that IL-1β induces ESE-1 expression in adult human articular chondrocytes further suggest a fundamental role for ESE-1 in cartilage degeneration and suppression of repair.
Recently, we have identified novel pathways that regulate chondrocyte terminal differentiation in the growth plate during endochondral ossification and also modulate chondrocyte phenotype in osteoarthritis. A microarray study of BMP-2-induced genes resulted in the discovery of a novel role for growth arrest and DNA damage inducible (GADD) 45β in chondrocyte survival and up-regulation of MMP-13 and Col10a1 gene expression during chondrogenesis [7,8]. The GADD45β gene product is implicated in the stress response, cell cycle arrest and apoptosis. GADD45α and γ, which are important mediators of tumor cell apoptosis, are not upregulated by BMP-2. In recent studies, we have begun to investigate the relative contributions of ESE-1 and GADD45β to OA initiation and progression, since both factors up-regulate MMP-13 gene expression and are expressed in early OA cartilage and at 3 to 6 months in the cho/+ mouse model, which develops OA-like pathology due to mutations that disrupt the collagen network [9]. We have also asked whether similar mechanisms regulate chondrocyte hypertrophy both during remodeling of the calcified cartilage matrix of the hypertrophic zone of the growth plate and during tidemark advancement in OA. Both processes share the features of chondrocyte hypertrophy, apoptosis and MMP-13-mediated remodeling. The distribution of immunostaining for GADD45β in early OA cartilage at sites peripheral to the lesion in chondrocyte clusters and in deep zone chondrocytes, along with our finding that it suppresses apoptosis, suggests that GADD45β may serve as a survival factor in activated chondrocytes, while promoting hypertrophy during tidemark advancement. An understanding of the relative contributions of the GADD45β and ESE-1 pathways to physiological cartilage homeostasis will provide understanding of the molecular mechanisms responsible for dysregulated cartilage remodeling in joint disease.

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18

GENETIC INFLUENCES ON OSTEOARTHRITIS: AN UPDATE

Tim D. Spector

A number of studies in the last few years have shown unequivocal evidence that at least 50% of the variance of OA in the hands, knees and hips is accounted for by genetic factors. These include classical twin studies of unselected populations as well as population based family studies and affected sib pair studies. A genetic effect on spinal disk degeneration, and spinal osteophytes, has also been demonstrated in twins using MRI data.

We have also shown in a recent twin study that the rate of progression of knee OA has a strong heritable basis.
Reports of significant associations for candidate genes for common forms of OA of the knee and hip now include over 50 genes – and over a dozen have now been replicated independently. These include VDR, ERG, CILP, Col2A1, AACT, BMP-2, FRZB, ADAM12, IL-1, IL-1-RA, ASPN, LRCH1, matrillin3, COMP, and OPG. Linkage studies using families and affected sib pairs have to date shown a number of significant replicated loci, especially areas on chromosome 2q and Chromosome 19.
OA is best studied genetically by dividing the phenotype into its constituent parts and by studying intermediate phenotypes, which may operate independently or together in clusters determined by pleiotropic genes. For example Cartilage loss at the fingers or knee medial compartment or serum COMP or bone turnover marker levels have all been shown to be heritable.
In conclusion, OA is a strongly genetic disease, which is likely to be a complex polygenic disorder that may differ genetically by gender site and race. We now have more than a dozen replicated genes and are close to being able to use these clinically for both diagnosis and clinically to predict progression and prognosis. Understanding how the individual genes influence the many intermediate processes is likely to be a fruitful avenue to provide insight into disease pathways and potential new drug targets.

19

TISSUE ENGINEERING BASED ON MUSCLE-DERIVED STEM CELLS: POTENTIAL APPLICATIONS FOR TISSUE REGENERATION

Johnny Huard

Summary: Members of my laboratory have isolated various populations of myogenic cells from the postnatal skeletal muscle of normal mice on the basis of the cells' adhesion characteristics, proliferation behavior, and myogenic and stem cell marker expression profiles. Although most of these cell populations have displayed characteristics similar to those of satellite cells, we also have identified a unique population of muscle-derived stem cells (MDSCs). MDSCs exhibit long-term proliferation and high self-renewal rates and can differentiate toward various lineages, both in vitro and in vivo. The transplantation of MDSCs, in contrast to that of other myogenic cells, has improved the efficiency of dystrophic muscle regeneration and the delivery of dystrophin to dystrophic muscle. The ability of MDSCs to proliferate in vivo for an extended period of time, combined with their capacity to exhibit self-renewal, multipotent differentiation, and transplantation. Recent studies performed by members of my laboratory have shown that transplantation of female MDSCs (F-FMSCs) rather than male MDSCs (M-MDSCs) significantly improves skeletal muscle regeneration despite the similar myogenic and stem cell marker expression by both cell types. I will explain the increased muscle regeneration efficiency exhibited by F-MDSCs. My presentation will also address the influence of environmental cues within dystrophic or injured skeletal muscle on the differentiation of MDSCs into fibrotic cells. I will discuss potential strategies by which to prevent scar tissue formation within injured muscle by blocking TGF-β1 activity. I then will discuss the use of MDSCs in gene therapy and tissue engineering applications designed to improve bone and articular cartilage healing through the genetic modification of MDSCs to express osteogenic proteins (BMP2 and -4) and the angiogenic factor VEGF. I will also outline in my presentation new results obtained with human muscle derived stem cells, which we believe will open new avenues by which researchers could use muscle stem cell-based gene therapy and tissue engineering to improve tissue regeneration.

20

NEW THERAPEUTIC STRATEGIES AND AGENTS WITH STRUCTURE MODIFYING POTENTIAL IN OSTEOARTHRITIS

Jean-Pierre Pelletier

Over the last decade, there have been several interesting advances in the treatment of osteoarthritis (OA). A clearer understanding of the pathophysiology of this disease has facilitated the development of new approaches for treatments aimed at specifically and effectively retarding the progress of the disease. Several new classes of molecules that inhibit one or more OA pathophysiological processes are under evaluation for their potential to alter the degenerative process.
Osteoarthritis can be described primarily as the degradation and loss of articular cartilage accompanied by subchondral bone remodeling, osteophyte formation, and synovial membrane inflammation. The most attractive recent findings are the data pointing to an association between inflammation and disease appearance and progression. There is significant interest in new agents that have the potential to reduce or stop the progression of structural changes observed in OA. Such agents offer great promise and are likely to lead to very significant changes in therapeutic approaches in the near future.
The use of NSAIDs or COX-2 selective inhibitors has shown that PGE_2 inhibition alone has not proven to delay the natural history of progressive OA. Other lipid mediators, including leukotrienes (LT), could play a major role in the development and persistence of the inflammatory process, and it is now clear that PG and LT have complementary effects. Recent studies have also revealed that LTB_4 potently stimulated the release of pro-inflammatory cytokines, such as IL-1β and TNF-α in human OA synovial membrane. Thus, the failure of NSAIDs to impact OA progression in these tissues could be due to the fact that inhibiting only the COX pathways leads to a shunt to LT production. Based on this concept, it is hypothesized that blocking production of both LT and PGE_2 could have a synergistic effect in achieving an optimal or a wider spectrum of anti-inflammatory activity.
Pro-inflammatory cytokines are likely responsible for some of the signs and symptoms as well as structural changes present in OA patients. There exists a number of ways by which the production or activity of cytokines could be reduced. These will be reviewed in brief. The action of cytokines can be reduced at the cell membrane level by decreasing the membrane receptor level or by the use of receptor antagonists or soluble receptors. Blocking the intracellular signaling pathways is another way to reduce the action of the cytokine.
The natural IL-1 receptor antagonist (IL-1Ra) is capable of reducing several cartilage catabolic processes that are IL-1β dependent. In vivo studies in animal models have demonstrated that the intra-articular injection of IL-1Ra could block the action of IL-1β and reduce disease progression. Data from early phase clinical trials in knee OA patients have also shown interesting symptomatic effects of IL-1Ra.
Another interesting target for controlling the activity of the IL-1 system is the IL-1 converting enzyme (ICE)/caspase-1, an enzyme which is responsible for the conversion of the proform of IL-1β into its active (mature) form. Usage of ICE inhibitor also represents another interesting potential target for the treatment of OA.
The role of proteases in the degradation of the extracellular matrix of cartilage in OA has been well documented, and metalloproteases (MMPs) are believed to play a major role in this process. Inhibition of the synthesis/activity of these enzymes as a treatment for OA has been the focus of intensive research. To date, the most promising strategy is still the use of chemical molecules that can block the activity of MMPs. A number of these compounds that have a broad range of MMP activities have already been tested in clinical trials. From these trials, we